Responses of *Coptotermes formosanus* and *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) to Three Types of Wood Rot Fungi Cultured on Different Substrates

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ABSTRACT This study examined the responses of two termite species, the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), to three types of wood decay fungi: a brown rot fungus, *Gloeophyllum trabeum* (Persoon: Fries) Murrill; a white rot fungus, *Phanerochaete chrysosporium* Burdsall; and a litter rot fungus, *Marasmiellus troyanus* (Murrill) Singer. We also examined the responses of termites to these three types of fungi grown on different substrates. For all three fungal species, both termite species showed a strong preference for fungus-infected sawdust over uninfected sawdust. In choice tests, both termite species preferred sawdust infected with either *M. troyanus* or *P. chrysosporium* over *G. trabeum.* However, termites did not show any preference for fungus-infected potato dextrose agar over uninfected potato dextrose agar. Tunneling activity of *C. formosanus* was greater in sand treated with methanol extracts of fungus-infected sawdust than in sand treated with extracts of uninfected sawdust. Because chemicals in the fungal extracts caused termites to tunnel further into treated sand than untreated sand, these chemicals could potentially be used to direct termite foraging toward bait stations in the field.

KEY WORDS *Coptotermes formosanus, Reticulitermes flavipes,* subterranean termites, wood decay fungi, interaction, foraging behavior

THE INTERACTION BETWEEN termites and wood decay fungi is complex. Subterranean termites prefer wood decayed by certain species of fungi and avoid wood decayed by others (Amburgey 1979, Becker 1976). Moreover, the response of termites to wood decayed by a particular fungus species varies with fungal strain, type of wood, decay rate of wood, and the interaction between the type of wood and decay rate of wood (Amburgey and Smythe 1977a, Lenz et al. 1980, Lenz et al. 1991). Decayed wood may provide nutritional benefits for termites by increasing the availability of nitrogen and other nutrients, breaking down toxic compounds in the wood, and enhancing the ability of termites to metabolize cellulose by chemically modifying the wood (Waller and La Fage 1987).

Many studies have documented that termites prefer wood decayed by certain species of brown rot fungi (reviewed by Amburgey 1979). Brown rot fungi use the hemicelluloses and cellulose of the cell wall and modify lignin by demethylation and oxidation without metabolizing it (Green and Highley 1997). Some species of brown rot fungi not only provide nutritional benefits for termites, but they also produce chemicals that act as cues to foraging termites. Workers of *Reticulitermes flavipes* (Kollar) orient shelter tubes toward wood blocks decayed by brown rot fungi, *Gloeophyllum trabeum* (Persoon: Fries) Murrill and *Poria incrassata* (Berkeley et Curtis) Burt (Amburgey and Smythe 1977c). Wood decayed by the brown-rot fungus, *G. trabeum*, elicits trail-following and aggregation behavior in *Reticulitermes* spp. (Esenther and Beal 1979, Rust et al. 1996). The compound, (Z,Z,E)-3, 6, 8-dodecatrien-1-ol, has been isolated and identified from wood decayed by *G. trabeum* (Smythe et al. 1967, Matsumura et al. 1968) and from whole body extracts of *Reticulitermes virginicus* (Banks) and *Coptotermes formosanus* Shiraki, suggesting that it may be the trail pheromone of these species (Matsumura et al. 1969, Tokoro et al. 1992).

Extracts of wood blocks decayed by other species of brown rot fungi, *Tyromyces palustris* Murrill, *Daedalea dickinsii* Yasuda, and *Serpula lacrymans* (Wulf.) S. F. Gray, also elicit trail-following activity by *C. formosanus* (Matsuo and Nishimoto 1974). However, bioassays of active fractions of *S. lacrymans* indicated that trail-following activity of termites to extracts of wood decayed by *S. lacrymans* was not due to (Z,Z,E)-3, 6, 8-dodecatrien-1-ol (Ohmura et al. 1995). Therefore,

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other chemicals produced by wood decay fungi may elicit trail-following activity in termites.

In contrast, several studies determined that wood decayed by some species of white rot fungi are avoided by termites (Amburgey and Beal 1977), and that wood decayed by the white rot fungus *Ganoderma applanatum* (Pers.) Pat. contains compounds that are toxic to termites (Amburgey 1979). However, other studies found that wood decayed by other species of white rot fungi are attractive to termites (French et al. 1981, Waller et al. 1987). All white rot fungi degrade lignin. Some white rot fungi degrade all cell wall components, while other white rot fungi can selectively remove lignin, leaving large concentrations of cellulose (Blanchette 1991). These differences in the mechanisms of decay used by different types of white rot fungi may account for the differences in termite responses to the decayed wood.

The substrate upon which a particular wood rot fungus is cultured appears to influence the production of chemicals that elicit trail-following activity by subterranean termites. For instance, trail-following activity by *R. virginicus* was not elicited by extracts of *G. trabeum* when it was grown on potato dextrose agar, unless an additional carbon source was added to the potato dextrose agar (Matsumura et al. 1976). When Amburgey and Smythe (1977b) cultured six isolates of *G. trabeum* on three types of nutrient media (2% malt agar, malt extract broth, and malt extract agar), these isolates differed in their capacity to elicit trail-following activity, and none of the isolates produced trail-following chemicals on all of the media tested.

There is tremendous diversity in the decay patterns among fungi found in nature (Blanchette 1991). Chemical modifications of the wood during the decay process vary depending on which type of fungus is involved. Therefore, it is necessary to identify how termites respond to different types of wood decay fungi, cultured under different conditions, and for different lengths of time to develop a greater understanding of the complex interactions between subterranean termites and wood decay fungi. The objectives of this study were to determine how the Formosan subterranean termite, *C. formosanus*, and the eastern subterranean termite, *R. flavipes*, responded to three types of wood decay fungi [a brown rot fungus, *G. trabeum;* a white rot fungus, *Phanerochaete chrysosporium* Burdsall; and a litter rot fungus, *Marasmiellus troyanus* (Murrill) Singer] and how differences in the substrate upon which the fungi were grown affected termite behavior. This study also examined how termites responded to extracts of *P. chrysosporium* grown on sawdust or pure cellulose to determine if *P. chrysosporium* produced chemicals which could act as cues to direct foraging termites toward a food source.

Although there are no published reports of associations of subterranean termites with litter rot fungi, any type of fungus that has the capacity to degrade lignin could potentially affect termite feeding and foraging behavior. Litter rot fungi refer to a group of lignin-degrading basidiomycetes that were collected in the leaf/plant litter and that are neither categorized as brown rot nor white rot fungi because they contain enzymes that are characteristic of both types. There are also no published reports of associations of subterranean termites with the white rot fungus, *P. chrysosporium.* Interactions between subterranean termites and white rot fungi are highly variable, ranging from attraction to repellency. *Phanerochaete chrysosporium* and *M. troyanus* were selected because these two species were readily available, have fast growth rates, and are easy to mass-produce in the laboratory on nutrient media. By comparing responses of *C. formosanus* and *R. flavipes* to *M. troyanus* and *P. chrysosporium* with their responses to *G. trabeum*, which is known to elicit trail-following behavior in both termite species, this study will increase our knowledge of the ecological relationships between termites and fungi.

Materials and Methods

Termite Collections and Maintenance. Termites were collected from field colonies in New Orleans, LA, using underground bucket traps (Su and Scheffrahn 1986) baited with blocks of spruce wood (*Picea* spp.). Termites were kept in the laboratory in 5.6-liter covered plastic boxes containing moist sand and blocks of spruce wood until they were used in experiments. *Coptotermes formosanus* and *R. flavipes* were identified to species using identification keys for soldiers (Scheffrahn and Su 1994). Voucher specimens of soldiers of each colony are stored in 70% alcohol at the Southern Regional Research Center, New Orleans, LA.

Fungus Cultures. Two isolates of the brown rot fungus, *G. trabeum* (Madison 539 and Madison 617), and one isolate of the white rot fungus, *P. chrysosporium* (#24775), were obtained from the American Type Culture Collection (ATTC, Manassas, VA). All bioassays with *G. trabeum* were conducted using Madison 539, unless stated otherwise. The litter rot fungus *M. troyanus* (TF-1867) was isolated from leaf litter in an abandoned oil refinery in Darrow, LA. Stock cultures of fungi were maintained at 8°C on malt extract agar slants.

Inoculation of Fungus-Infected Material. Experiments were conducted using the following fungus-infected substrates: spruce sawdust, soybean hulls, sugarcane bagasse, potato dextrose agar, and pure cellulose (Solka-Floc; F.S. & D., Urbana OH). Soybean hulls and sugarcane bagasse are agricultural waste products which were previously being used as substrates for *M. troyanus* and *P. chrysosporium* because they are inexpensive, readily available substrates that are suitable for fungal growth.

Soybean hulls and sugarcane bagasse were inoculated with fungi using the following protocol: 350 g of the substrate was placed in an autoclavable polypropylene bag (36 by 61 cm) (Unicorn Importers, Commerce, TX) with a single 0.2-micron filter (7.6 by 25.4 cm). The bag was heat sealed, and a 2-cm slit, loosely covered by autoclave tape, was made in the bag. The bag was autoclaved, vent-side up, for 60 min on each of two consecutive days. After cooling to room tem-

perature, the substrate was inoculated with potato dextrose agar plugs of the fungi in 625 ml sterile water. The slits through which the substrate was inoculated were sealed with one layer of autoclave tape and one of glass fiber tape. The inoculated bags were placed in incubators set at a temperature of 25°C with a photoperiod of 12:12 (L:D) h. The same methods were used to prepare control bags, except that the potato dextrose agar plugs were uninfected.

The fungus-infected spruce sawdust was prepared using the same protocol described previously. After cooling to room temperature, the substrate was inoculated with either *M. troyanus* (20 plugs taken from potato dextrose agar plates in 625 ml sterile, Milli-Q filtered water), *P. chrysosporium* mycelium mats (grown in shake flasks cultures of Sabouraud dextrose broth over 7 d, filtered, and rinsed with sterile, Milli-Q filtered water) at amounts of 9.3 g and 18.6 g of mycelium in 625 ml Sabouraud dextrose broth, or *G. trabeum* mycelium mats (grown in shake flasks cultures of Sabouraud dextrose broth over 16 d, filtered, and rinsed with sterile, Milli-Q filtered water) at amounts of 4.3 and 8.5 g of mycelium in 625 ml of sterile, Milli-Q filtered water. The methods used to inoculate the sawdust with the three fungi differed because there were substantial differences in the growth rates of the three fungal species on sawdust. Because it was extremely difficult to infect the spruce sawdust with *P. chrysosporium* and *G. trabeum* using the potato dextrose agar plugs, mycelium mats of these two species were grown in a nutrient broth and then the sawdust was inoculated with these mycelium mats. In contrast, mycelium of *M. troyanus* rapidly colonized sawdust from the potato dextrose agar plugs. Because the growth rate of *P. chrysosporium* in the broth was much faster than that of *G. trabeum*, there were differences in the length of time each fungus species was grown in the broth. Bags of uninfected sawdust were prepared using the same procedure followed for preparing bags of fungus-infected sawdust to serve as controls.

A bag containing 250 g of pure cellulose, Solka-Floc, was inoculated with 52.73 g of filtered mycelium of *P. chrysosporium* (grown in shake flasks cultures of Sabouraud dextrose broth over 7 d, filtered, and rinsed with sterile, Milli-Q filtered water) following the same procedure described previously. A bag containing 100 g of sterile, uninfected Solka-Floc was used as a control.

Potato-dextrose agar in plastic petri dishes (100 by 15 mm) was inoculated with fungi, and placed in an incubator at 25°C with a photoperiod of 12:12 (L:D) h. Fungus cultures were grown on potato dextrose agar for 1–2 wk until fungal hyphae covered the surface of the petri dish.

Fungal Extracts. To determine if the response of termites to fungus-infected material was caused by chemicals produced by the fungi, tests were conducted using methanol extracts of fungus-infected and uninfected material. Fungus-infected sawdust was freeze-dried using a Virtis Freezemobile 12ES freeze dryer. The resulting dry sawdust was stirred vigorously with methanol (10 ml/g of fungus-infected sawdust) at room temperature for ≈18 h. The methanol filtrate was collected from the sawdust by vacuum filtration through Whatman #1 filter paper (Whatman, Hillsboro, OR), and the sawdust was washed with three 25-ml aliquots of methanol. The organic filtrates were combined, and methanol was removed by rotary evaporation at 40°C, followed by removal of residual methanol at room temperature under a gentle stream of nitrogen. An average of 50 mg of essential oil extractives was obtained for each gram of fungus-infected sawdust extracted. Fungus-infected Solka-Floc was extracted with methanol using the same method as the sawdust.

Termite Responses to Fungus-Infected Material. Bioassays were conducted using Rubbermaid storage containers (14.5 by 8.5 by 4 cm) (Consolidated Plastics, Twinsburg, OH). Each container contained 100 g of sand (Standard Sand and Silica Company, Davenport, FL) moistened with 20 ml of water. Each container had a 2-cm-diameter hole on each side. A 14-ml (17 by 100 mm) polystyrene round-bottom Falcon test tube (Becton Dickinson, Franklin Lakes, NJ) was inserted into each hole and sealed in place using a glue gun. There were two treatment tubes and two control tubes connected to each container (Fig. 1). The position of treatment and control tubes was rotated between replicates to preclude any positional effects. Each tube was filled with a 10-ml volume of material. Treatment tubes were filled with fungus-infected material and control tubes were filled with uninfected material. If necessary, water was added to the material to equalize moisture levels between treatment and control tubes.

For direct comparisons of termite responses to sawdust infected with two fungus species or two isolates of the same fungus species, two tubes were filled with sawdust infected with one fungal species (isolate), and two tubes were filled with sawdust infected with another fungal species (isolate). For direct comparisons of termite responses to sawdust decayed by a particular species of fungus over different lengths of time, two tubes filled with sawdust decayed by a fungus for a certain number of weeks were paired with two tubes filled with sawdust decayed by the same species of fungus for a different number of weeks.

For experiments where fungi were grown on potato dextrose agar, the potato dextrose agar was mixed with sand so that the effect of fungus-infected potato dextrose agar on the tunneling behavior of termites could be determined. The potato dextrose agar in each petri dish was cut into quarters and each quarter was mixed thoroughly with sand. Each tube was filled with a 10-ml volume of the potato dextrose agar–sand mixture so that each tube contained one-quarter of the potato dextrose agar in a petri dish. Treatment tubes were filled with a fungus-infected potato dextrose agar–sand mixture and control tubes were filled with an uninfected potato dextrose agar–sand mixture.

In each container, 200 termites (*C. formosanus:* 180 workers and 20 soldiers; *R. flavipes:* 196 workers and four soldiers) were placed in the center of each con-

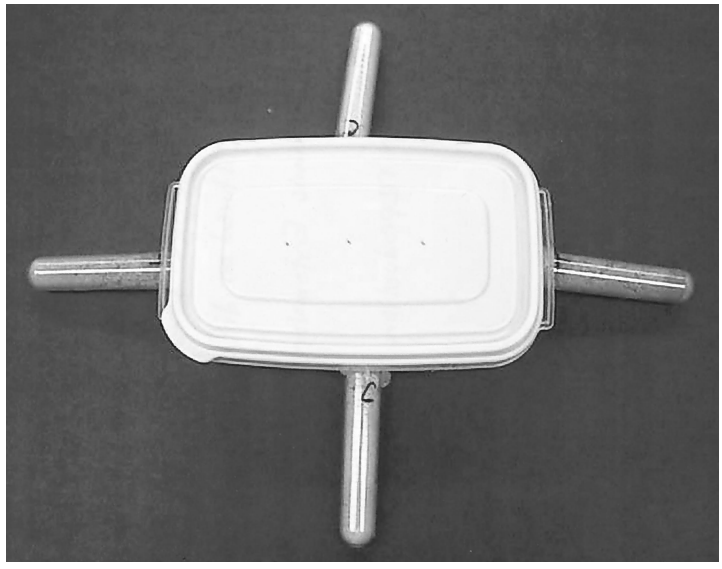

Fig. 1. Bioassay unit composed of a plastic container (14.5 by 8.5 by 4 cm) filled with 100 g of moistened sand and connected to four 14-ml polystyrene round-bottom Falcon test tubes (17 by 100 mm) containing either fungus-infected (two tubes) or uninfected material (two tubes).

tainer. In some replicates of *R. flavipes*, there were fewer than four soldiers present because numbers of soldiers collected from some traps were extremely low. For each experiment, two colonies of each termite species were used, with at least five replicates from each colony. Containers were placed in an unlit environmental chamber (28°C, 97% RH) for 18–22 h. After which, each tube was capped with a rubber stopper and removed from the container. The number of termites within each tube was counted.

Termite Responses to Fungal Extracts. A bioassay was developed to determine how fungal extracts affected tunneling activity in treated and control sand. The testing device was composed of a clear polystyrene cylindrical screwtop container (9 cm high by 7 cm diameter, Consolidated Plastics, Twinsburg, OH) with a 5-cm length of Tygon tubing (0.8 cm i.d.) inserted through a hole on the lower side of the container and sealed in place with a glue gun. A plastic Y-tube (stem, 3 cm; arms, 3 cm; diameter, 1 cm) was attached to the other end of the Tygon tube and another 5-cm length of Tygon tubing (0.8 cm i.d.) was attached to each arm of the Y-tube. A 1-ml disposable pipette tip (length: 7 cm; diameter: 1 cm at wide end) was attached to the other end of the Tygon tubing

Fig. 2. Bioassay apparatus to evaluate termite tunneling activity in 2 g of treated or control sand within dual pipette tips (1 ml) connected via Tygon tubing (0.8 cm i.d.) and a plastic Y-tube (stem: 3 cm; arms: 3 cm, diameter: 1 cm) to a clear, polystyrene container (9 cm high by 7 cm diameter).

Table 1. Mean ± SEM number of *C. formosanus* or *R. flavipes* in tubes filled with fungus-infected or uninfected material after 18 to 22-h exposure

Termite species	Fungus species	Substrate	No. of Replicates	No. of termites in tubes	
				Fungus-infected	Uninfected
C.f.	*G. t.*	Soybean hulls	20	20.2 ± 5.7	3.4 ± 1.0**
C.f.	*M. t.*	Soybean hulls	20	30.0 ± 6.8	1.9 ± 0.6**
C.f.	*M. t.*	Bagasse	16	29.1 ± 9.6	6.9 ± 1.8*
C.f.	*G. t.*	Sawdust	10	15.7 ± 4.8	1.2 ± 0.7*
R.f.	*G. t.*	Sawdust	10	92.2 ± 20.2	0.0 ± 0.0**
C.f.	*M. t.*	Sawdust	10	42.7 ± 11.0	0.200 ± 0.1**
R.f.	*M. t.*	Sawdust	10	79.3 ± 11.9	0.0 ± 0.0**
C.f.	*P. c.*	Sawdust	10	42.4 ± 8.8	0.400 ± 0.2**
R.f.	*P. c.*	Sawdust	20	88.2 ± 10.9	0.35 ± 0.2**

C. f., Coptotermes formosanus; R. f., Reticulitermes flavipes; G. t, Gloeophyllum trabeum; M. t., Marasmiellus troyanus; P. c., Phanerochaete chrysosporium.

*, **, Significant t values at $P \leq 0.05$, $P \leq 0.01$, respectively.

(Fig. 2). A thin layer of moist sand was placed on the bottom of the container. Termites were able to move freely from the container into the tubing. In each container, 200 *C. formosanus* workers were placed in the center of the container. For each experiment, two colonies were used, with 10 replicates from each colony.

Experiments were conducted to determine if fungal extracts could affect the tunneling behavior of termites by testing methanol extracts of material infected with *P. chyrsosporium.* Experiments were conducted using extracts of fungus-infected sawdust and fungus-infected Solka-Floc. If termites responded to extracts of fungus grown on a defined chemical media, such as pure cellulose (Solka-Floc), it would simplify the process of isolating and identifying chemicals produced by fungi that elicit directed foraging behavior.

For each experiment, 10 ml of a methanol extract of fungus-infected sawdust (Solka-Floc) was added to 40 g sand in a glass beaker and 10 ml of a methanol extract of uninfected sawdust (Solka-Floc) was added to 40 g of sand in another glass beaker. Extract-treated sand was allowed to air dry until solvent completely evaporated, and then 2 g sand was placed in each pipette tip and moistened with 400 microliters of distilled water. For each replicate, one arm of the Y-tube was connected to a pipette tip filled with sand treated with a methanol extract of fungus-infected sawdust and the other pipette tip was filled with sand treated with a methanol extract of uninfected sawdust. The position of the treatment and control tips on the arms of the Y-tube was rotated between replicates to preclude any positional effects. Each pipette tip was marked on the outer surface with a permanent marker at a distance of 2 cm from the narrow end of the pipette tip. Tunneling activity was observed until termites reached this mark. As soon as termites reached this mark in one of the tips attached to each Y-tube, both tips were removed and the number of termites in each tip were counted. For any replicates in which termites did not reach this mark in either tip within 5 h, both tips were removed after 5 h, and the number of termites in each tip was counted. These tests were conducted in ambient conditions in the laboratory. For each experiment, data were recorded on the number of treatment and control tips which termites tunneled through completely (termites reached the mark) and the number of termites in treatment and control tips.

Statistical Analysis. To compare termite preferences for fungus-infected or uninfected material, data on the number of termites in treatment and control tubes after 18–22 h were analyzed using a t-test for matched pairs (SYSTAT 1998). To compare the tunneling activity of termites in sand treated with extracts of fungus-infected or uninfected material, data on the number of treatment and control tips which termites tunneled through completely were analyzed using the binomial distribution (Sign test) and data on numbers

Table 2. Mean ± SEM number of *C. formosanus* or *R. flavipes* in test tubes containing fungus-infected sawdust in a choice test after 18 to 22-h exposure

Termite species	Fungus species in tube A	Fungus species in tube B	No. of termites in	
			Tube A	Tube B
C. f.	*M. t.*	*G. t.*	45.4 ± 13.9	8.5 ± 4.1*
R. f.	*M. t.*	*G. t.*	78.5 ± 15.8	12.7 ± 7.8*
C. f.	*P. c.*	*G. t.*	73.3 ± 17.1	16.4 ± 9.5*
R. f.	*P. c.*	*G. t.*	71.7 ± 11.6	17.5 ± 9.2*
C. f.	*M. t.*	*P. c.*	15.6 ± 4.7	46.3 ± 15.4
R. f.	*M. t.*	*P. c.*	42.6 ± 14.0	49.2 ± 19.6
C. f.	*G. t.* (539)	*G. t.* (617)	15.4 ± 6.1	7.5 ± 4.1
R. f.	*G. t.* (539)	*G. t.* (617)	47.5 ± 14.2	23.4 ± 11.8

C. f., Coptotermes formosanus; R. f., Reticulitermes flavipes; G. t., Gloeophyllum trabeum; M. t., Marasmiellus troyanus; P. c., Phanerochaete chrysosporium.

*, Significant t values at $P \leq 0.05$.

Table 3. Mean ± SEM number of *C. formosanus* or *R. flavipes* in test tubes containing fungus-infected sawdust in a choice test after 18 to 22-h exposure

Termite species	Fungus species	No. of weeks fungus grown in		No. of termites in	
		Tube A	Tube B	Tube A	Tube B
C. f.	*M. t.*	3	6	48.1 ± 9.7	4.1 ± 2.2**
R. f.	*M. t.*	3	6	24.6 ± 10.8	22.8 ± 10.0
C. f.	*G. t.*	3	6	5.9 ± 2.7	9.4 ± 2.9
R. f.	*G. t.*	3	6	54.9 ± 15.5	20.2 ± 8.8
C. f.	*P. c.*	3	6	10.9 ± 5.6	10.5 ± 3.8
R. f.	*P. c.*	3	6	19.5 ± 7.7	55.4 ± 14.5
C. f.	*P. c.*	3	12	5.6 ± 1.8	6.8 ± 2.2
R. f.	*P. c.*	3	12	43.8 ± 14.4	14.5 ± 6.9
C. f.	*G. t.*	3	12	81.7 ± 15.2	3.0 ± 1.5**
R. f.	*G. t.*	6	12	44.2 ± 12.6	39.3 ± 14.9
R. f.	*M. t.*	6	12	36.2 ± 10.3	28.0 ± 8.0

C. f., Coptotermes formosanus; R. f., Reticulitermes flavipes; G. t., Gloeophyllum trabeum; M. t., Marasmiellus troyanus; P. c., Phanerochaete chrysosporium.

*, **, Significant t values at $P \leq 0.05$, $P \leq 0.01$, respectively.

of termites in treatment and control tips were analyzed using a t-test for matched pairs (SYSTAT 1998).

Results

Termite Responses to Fungus-Infected Material. In tests where fungi were cultured on various cellulose-containing substrates, numbers of *C. formosanus* in tubes with fungus-infected material were significantly greater than numbers in control tubes for tests using soybean hulls infected with both *G. trabeum* and *M. troyanus* and in tests using sugarcane bagasse infected with *M. troyanus* (Table 1). Numbers of both *C. formosanus* and *R. flavipes* were much greater in tubes containing sawdust infected with either *G. trabeum*, *M. troyanus* or *P. chrysosporium* than in control tubes (Table 1). In fact, virtually all of the termites in tubes containing sawdust were located in tubes containing fungus-infected sawdust.

In tests directly comparing termite responses to sawdust infected with two species of fungus, numbers of both *C. formosanus* and *R. flavipes* were significantly greater in tubes containing sawdust infected with either *M. troyanus* or *P. chrysosporium* than in tubes containing sawdust infected with *G. trabeum* (Table 2). There were no significant differences in the responses of either termite species to *M. troyanus* compared with *P. chrysosporium* or between the two isolates of *G. trabeum*, Madison 539 and Madison 617.

In tests comparing the responses of termites to fungus-infected sawdust after different lengths of time, there were differences in the responses of the two termite species. When *M. troyanus*-infected sawdust that had been decayed for 3 wk was compared with sawdust that had been decayed for 6 wk, *C. formosanus* showed a significant preference for sawdust decayed for 3 wk, but *R. flavipes* showed no preference for sawdust decayed for 3 wk versus sawdust decayed for 6 wk. Neither termite species showed any preference for sawdust decayed by *P. chrysosporium* for 3 wk versus sawdust decayed for 6 or 12 wk; however, *C. formosanus* showed a strong preference for sawdust decayed by *G. trabeum* for 3 wk versus sawdust decayed by *G. trabeum* for 12 wk (Table 3). Neither termite species showed any preference for fungus-infected potato dextrose agar over uninfected potato dextrose agar (Table 4). In the test with *G. trabeum*, numbers of *C. formosanus* were significantly greater in control tubes than in treatment tubes.

Termite Responses to Fungal Extracts. When extracts of *P. chrysosporium*-infected sawdust had a concentration of either 1.5 mg/g sand or 1.75 mg/g sand, *C. formosanus* tunneled completely through the sand treated with fungal extracts before tunneling through the sand treated with extracts of uninfected sawdust (Table 5). Numbers of termites in tips with treated sand were significantly greater than numbers in control tips for all three concentrations. However, there was no difference in the tunneling activity or in the number of termites in treatment and control tips when the sand was treated with a methanol extract of *P. chrysosporium*-infected Solka-Floc compared with sand treated with a methanol extract of uninfected Solka-Floc (Table 5).

Discussion

Both termite species strongly preferred sawdust infected with *G. trabeum*, *P. chrysosporium*, and *M. troya-*

Table 4. Mean ± SEM number of *C. formosanus* and *R. flavipes* in test tubes containing sand mixed with fungus-infected PDA or uninfected PDA after 18 to 22-h exposure

Termite species	Fungus species	No. of termites in tubes	
		Fungus-infected	Uninfected
C. f.	*M. t.*	15.8 ± 6.3	18.7 ± 4.5
R. f.	*M. t.*	39.2 ± 14.7	28.0 ± 12.3
C. f.	*G. t.*	7.2 ± 2.1	15.7 ± 3.2*
R. f.	*G. t.*	35.5 ± 15.2	18.7 ± 8.2
C. f.	*P. c.*	9.2 ± 4.9	31.9 ± 9.6
R. f.	*P. c.*	44.1 ± 21.6	16.2 ± 10.5

C. f., Coptotermes formosanus, R. f., Reticulitermes flavipes; G. t., Gloeophyllum trabeum; M. t., Marasmiellus troyanus; P. c., Phanerochaete chrysosporium.

*, Significant t values at $P \leq 0.05$.

Table 5. Tunneling behavior of *C. formosanus* in pipette tips filled with sand treated with a methanol extract of either sawdust or Solka-Floc infected with the fungus *P. chrysosporium* versus sand treated with a methanol extract of either uninfected sawdust or Solka-Floc over a 5-h exposure period

Substrate (no. of weeks fungus grown on substrate)	Extract concen[a] (mg/g sand)	No. of tips termites tunneled through completely		Mean no. of termites in tips	
		Treated	Control	Treatment	Control
Sawdust (4 wk)[b]	0.75	11	7	13.8 ± 2.0	5.8 ± 1.2*
Sawdust (4 wk)[b]	1.5	20	0**	18.8 ± 1.0	6.9 ± 0.9**
Sawdust (8 wk)[b]	1.75	16	0**	18.6 ± 2.0	10.1 ± 1.7**
Solka-Floc (4 wk)	1.88	13	7	23.4 ± 2.3	22.5 ± 1.1

*, **, Significant t values at $P \leq 0.05$, $P \leq 0.01$, respectively.
[a] An average of 50 mg of essential oil extractives was obtained for each gram of fungus-infected sawdust extracted.
[b] Bioassays conducted concurrently.

nus to uninfected sawdust. Because these fungi represent three different types of wood rot fungi, these results indicate that under certain conditions, *C. formosanus* and *R. flavipes* may prefer wood decayed by a wide variety of species of wood rot fungi over sound wood. Moreover, this study is the first examination of termite interactions with either *P. chrysosporium* or *M. troyanus.* Neither of these species had been reported to be associated with termites. However, any fungal species that has the capacity to degrade lignin could potentially influence the foraging behavior of subterranean termites in the field. Therefore, research on interactions between termites and lignin-degrading fungi should not necessarily focus solely on those fungus species that have actually been collected on termite-infested wood.

Much of the literature on the association between subterranean termites and wood decay fungi has focused on *G. trabeum.* In choice tests with fungus-infected sawdust, both termite species preferred *M. troyanus* and *P. chrysosporium* over *G. trabeum.* Because the rate of growth of *G. trabeum* is slower than that of the other two fungus species, these results could merely reflect differences in the growth rates of the three fungus species on sawdust. Also, another study determined that the *G. trabeum* isolate Madison 5096–15 was more attractive to *R. flavipes* than the Madison 617 isolate (Amburgey and Smythe 1977a). In the current study, responses of termites to isolates Madison 617 and Madison 539 were similar. Responses of termites to *G. trabeum* may have been stronger if a different isolate had been used. In any case, this study demonstrated that there are other species of wood rot fungi that may produce chemicals that act as cues to foraging termites. These chemicals could potentially be used to direct termite foraging toward bait stations in the field.

There were differences in the responses of the two termite species to sawdust infected with fungi for different lengths of time. In two cases, *C. formosanus* preferred sawdust infected for only 3 wk versus 6 or 12 wk, whereas *R. flavipes* showed no difference in response to sawdust decayed over the 12-wk period. In the field, *R. flavipes* is more likely to be associated with decaying wood and *C. formosanus* is more likely to be associated with sound wood (Lenz et al. 1991). In laboratory studies using wood blocks decayed by *G. trabeum*, *R. flavipes* showed a pronounced increase in feeding and survival as the rate of decay increased from sound to moderately decayed and a slight increase from moderately decayed to heavily decayed, whereas *C. formosanus* showed a much less pronounced increase in feeding and survival on moderately decayed wood compared with sound wood, and no increase from moderately decayed to heavily decayed (Lenz et al. 1991).

Neither termite species showed a preference for fungi grown on potato dextrose agar alone. Therefore, termites are not responding to the wood rot fungi by themselves, but rather they are apparently responding to chemicals produced by these fungi when they are metabolizing cellulosic materials. However, when *P. chrysosporium* was grown on pure cellulose, *C. formosanus* did not respond to sand treated with fungal extracts. It is possible that chemicals that affect termite behavior are produced by fungi during the process of breaking down lignin or other wood components.

Tunneling behavior of *C. formosanus* in sand treated with methanol extracts of fungus-infected sawdust clearly demonstrated that chemicals produced by fungi growing on sawdust influenced termite behavior. Other studies determined that chemicals produced by fungus-infected wood elicited trail-following behavior (Esenther and Beal 1979). Because these chemicals elicit trail-following activity and directed foraging behavior, they could potentially be used to improve the efficacy of baits to control termite populations. A jellied bait formulation using fungus-infected sawdust, bagasse dust, potato dextrose agar, and mirex has been developed for control of *C. formosanus* in China (Gao 1985). However, because decay rate influences the attractiveness of fungus-infected sawdust to termites, the use of specific chemicals in a bait matrix or in the soil surrounding the bait may prove to be a more effective method than using fungus-infected material. Further studies are necessary to isolate and identify the chemicals causing the behavioral response and to determine if the different types of fungi are producing chemicals other than (Z,Z,E)-3, 6, 8-dodecatrien-1-ol that elicit trail-following behavior. Also, studies are necessary to determine how the production of these chemicals changes when a particular fungus species is grown on different substrates.

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